Cannabis-dependent adolescents show differences in global reward-associated network topology: a functional connectomics approach.

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Abstract

Adolescence may be a period of increased vulnerability to the onset of drug misuse and addiction due to changes in developing brain networks that support cognitive and reward processing. Cannabis is a widely misused illicit drug in adolescence which can lead to dependence and alterations in reward-related neural functioning. Concerns exist that cannabis-related alterations in these reward networks in adolescence may sensitize behaviour towards all forms of reward that increases the risk of further drug use. Taking a functional connectomics approach, we compared an acutely abstinent adolescent cannabis-dependent (CAN) group with adolescent controls (CON) on global measures of network topology associated with anticipation on a monetary incentive delay task. In the presence of overall superior accuracy, the CAN group exhibited superior global connectivity (clustering coefficient, efficiency, characteristic path length) during monetary gain anticipation compared to the CON group. Additional analyses showed that the CAN group exhibited significantly greater connectivity strength during monetary gain anticipation across a sub-network that included mesocorticolimbic nodes involving both inter and intra hemispheric connections. We discuss how these differences in reward-associated connectivity may allude to subtle functional alterations in network architecture in adolescent cannabis-dependence that could enhance the motivation for non-drug reward during acute abstinence.

Introduction

Adolescence is a period of substantial neurobiological development. Adolescents exhibit a number of psychological traits, such as risk-taking and reward-seeking (Steinberg, 2008; van Hemel-Ruiter et al., 2012), the emergence of which may reflect the relatively early functional development of brain networks related to these behaviours (Casey et al., 2008; Galvan et al., 2006). As such, the period of adolescence may confer an increased vulnerability, in some, to the onset of drug misuse and addiction (Chambers et al., 2003; Nixon et al., 2010), due to developmental changes in brain networks (Schneider et al., 2012; Stice et al., 2013; Whelan et al., 2012). Cannabis is a widely misused illicit drug in adolescence (Eaton et al., 2006), which can lead to dependence (Chen et al., 2005) and alterations in reward-related neural functioning (Ellgren et al., 2008; Jager et al., 2013). Significantly, cannabis use in adolescence may enhance the motivation to use other illicit drugs of abuse (Lopez-Quintero et al., 2018; Taylor et al., 2017), possibly due to perturbed alterations in brain connectivity (Manza et al., 2018; Orr et al., 2013; Prashad et al., 2018). Therefore, probing functional connectivity related to motivation and reward-seeking may elucidate important functional alterations in network architecture that are provisions for the maintenance of addiction in adolescence.

The widespread spatial distribution of functional abnormalities reported in clinical populations during functional MRI studies may suggest that there are extensive disruptions to network functioning across the entire brain. The integrity of brain networks can be probed by extracting graph characteristics that relate to their topological functioning (Bullmore et al., 2009a), a method that has already been used to capture properties of resting state connectivity in psychiatric populations

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(Bassett et al., 2008; Luo et al., 2015; Ye et al., 2015), including addiction (Jiang et al., 2013; Morris et al., 2018; Sjoerds et al., 2017; Tschernegg et al., 2013; Yuan et al., 2010). The properties of resting state networks cannot fully elucidate the characteristics of brain connectivity related to specific types of behaviour, however, and thus require a behavioural-based approach to characterising network functioning. Indeed, this behavioural-based connectivity approach has been utilized in various clinical psychiatric populations (Fornito et al., 2011; Manelis et al., 2016; Ray et al., 2017), providing a more precise evaluation of functional connectivity across networks that relate to specific behavioural processes. Importantly, processing related to different behaviours in addiction populations may also be evaluated across brain networks that could explicate alterations in functional connectivity that maintain addiction.

There is some evidence that cannabis use may sensitize brain network functioning (Prashad et al., 2018), particularly in regions that respond under conditions of non-drug reward-seeking (Filbey et al., 2013b; Jager et al., 2013; Nestor et al., 2010). While this evidence appears to contravene more widespread findings of deficits in non-drug reward processing in addiction populations (Luijten et al., 2017), these studies have probed regional differences using analytic methods that cannot capture the features that represent the brain as a single functioning network. Therefore, the current study compared a group of cannabis-dependent adolescents in acute abstinence against a group of adolescent controls on measures of network functioning associated with anticipation on a monetary incentive delay task. We hypothesized that 1) the cannabis group would elicit markers of increased global network functioning during the anticipation of non-drug rewards and 2) that these markers would be related to life-time cannabis use that

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may suggest a sensitization of network functioning related to non-drug reward processing.

Material and Methods

Participants

Eighteen cannabis-dependent adolescents (CAN: mean age 16.50 ± 0.23; 17 males, 1 female) and 18 comparison healthy adolescent controls (CON: mean age 16.11 ± 0.41; 17 males, 1 female) completed the current study. Participants were screened for past or present histories of psychiatric or neurological illness. Information pertaining to any form of treatment (counselling, psychological and psychiatric), past or present, was carefully detailed, with any potential participant describing any major lifetime psychiatric event or brain injury (e.g. head trauma resulting in a loss of consciousness, seizure or stroke) considered ineligible for the study. Participants were also considered ineligible if they reported any familial psychiatric history (i.e. sibling, parent or grandparent). The CAN group were recruited from several drug treatment centres in Dublin, Ireland, and were being treated for current cannabis dependence at the time of testing. Diagnosis of cannabis dependence in the CAN group was confirmed by a fully qualified adolescent psychiatrist who administered the World Health Organization's Composite International Diagnostic Interview (CIDI), which is based on diagnostic criteria from the Diagnostic and Statistical Manual on Mental Disorders version IV(Kessler et al., 1998). The CIDI was also administered to exclude participants from either group who met criteria for any Axis I psychiatric disorder other than nicotine dependence. The CAN group were asked to refrain from cannabis use the night before the scan in order to avoid the potential confounding effects of acute

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Delta-9-tetrahydrocannabinol (THC) intoxication. All participants provided a urine sample which tested for the presence of amphetamines, barbiturates, benzodiazepines, cocaine, methadone and opiates, THC, and tricyclic antidepressants (Triage® Drugs of Abuse Panel, Inverness Medical UK Limited). All members of the CAN group tested positive for THC only. The CON group were recruited through university mailing lists. Within the CON group, four had reported previous minimal use of cannabis, three of which had reported smoking cannabis in the preceding month. Further information about the CON group can be found in Behan (Behan et al., 2014), who used the same cohort to test for neural differences in cognitive control against a slightly smaller adolescent CAN group using a go/no-go task. All participants provided written informed consent, and the study was conducted in accordance with the Declaration of Helsinki. The study was ethically approved by the School of Psychology at Trinity College Dublin, Ireland.

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Questionnaires

As per Behan (Behan et al., 2014), participants were administered the Wide Range Achievement Test 4 (WRAT4) to assess basic literacy and arithmetic (Wilkinson et al., 2006), the scores of which were subsequently standardised with respect to each participant's age. The short (21 item) form of the Depression anxiety and stress scale (DASS) was administered to all participants to assess psychological wellbeing during the week prior to study participation (Lovibond et al., 1995). Current and life-time substance use was measured through the completion of a 28-day time-line follow back calendar, and the completion of a general drug use questionnaire (Hibell et al., 2003). Drug use and abuse was specifically assessed in the both groups using modules taken from the WHO CIDI-SF (Kessler et al., 1998).

The CAN group reported using cannabis for the first time, on average, before thirteen years of age (mean 12.89 ± 0.24; range=11-15), with a mean consumption of 3830 (±1014.96; range=400-14600) life-time cannabis joints.

Monetary Incentive Delay Task (MID)

We used a "monetary incentive delay task" (MID), which was based on that originally employed by Knutson (Knutson et al., 2001), and which was originally used to assess the neural correlates of reward processing in cannabis users (Nestor et al., 2010). While being scanned participants performed the MID task, during which they anticipated potential monetary gain, loss or no potential monetary outcome. During each trial, participants viewed one of three coloured squares (cue) that indicated the potential to gain fifty cent (green square), lose fifty cent (red square) or experience no financial outcome (blue square - here referred to as the neutral condition) following their response to an upcoming visual target (see Supplementary Fig 1). Each cue was presented for a variable duration (2-8 sec), after which participants made a button press response upon the presentation of a visual target (star located within a circle). Participants received feedback (1500 ms) following their response to the visual target, after which there was an end fixation period (2-8 sec) before the commencement of the next trial. Responses to the visual target falling within ("hits") or outside ("misses") a 400ms response deadline received feedback appropriate for that particular trial. We chose this 400 ms time frame in order to yield accuracy levels at ~50%, which would serve to maintain the participant's interest in the task. Therefore, participants had four hundred milliseconds to respond to the visual target in order to be successful on a gain, loss or neutral trial. Each run contained nine gain, nine loss, and nine neutral trials.

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Therefore, there were a total of 27 trials in each condition, with each trial lasting between six and eighteen seconds. The MID was composed of three runs, with each run lasting 320 seconds. The order of trials within each run was randomised. Dependent measures derived from the data included mean percentage accuracy and reaction time for the gain, loss and neutral conditions. The task was programmed and run using E-Prime (Psychology Software Tools, Pittsburgh, USA).

Functional MRI (fMRI) Data Acquisition

All scanning was conducted on a Philips Intera Achieva 3.0 Tesla MR system (Best, The Netherlands) equipped with a mirror that reflected the visual display, which was projected onto a panel placed behind the participants' head outside the magnet. The mirror was mounted on the head coil in each participant's line of vision. Each scanning sequence began with a reference scan to resolve sensitivity variations. A parallel sensitivity encoding (SENSE) approach with a reduction factor of 2 was utilised for all T1-weighted image acquisitions (Pruessmann et al., 1999). 180 high-resolution T1- weighted anatomic MPRAGE axial images (FOV 230 mm, thickness 0.9 mm, voxel size 0.9×0.9×0.9) were then acquired (total duration 325 seconds), to allow subsequent activation localization and spatial normalization. Functional data were acquired using a T2* weighted echo-planar imaging sequence collecting 32 non-contiguous (10% gap) 3.5 mm axial slices covering the entire brain (TE=35 ms, TR=2000 ms, FOV 224 mm, 64×64 mm matrix size in Fourier space). Functional scans had a total duration of 320 seconds per run.

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fMRI Data analyses

Data pre-processing and statistical analysis were conducted using FEAT (fMRI Expert Analysis Tool) from the FMRIB Software Library (FSL 5.0.9, www.fmrib.ox.ac.uk/fsl). Statistical pre-processing was as follows: motion correction utilizing FMRIB's Linear Image Registration Tool (MCFLIRT); non-brain matter removal using Brain Extraction Tool (BET); spatial smoothing with a 6-mm full-width half maximum Gaussian kernel; mean-based intensity normalization; nonlinear high-pass temporal filtering (Gaussian-weighted least squares straight line fit, with sigma = 25.0 seconds).

For each participant, first level whole-brain mixed-effects analyses were performed by modelling the MID anticipation periods (i.e., gain, loss and neutral) as explanatory variables within the context of the general linear model on a voxel-by-voxel basis (variable boxcar functions for the anticipation period regressors were convolved with the haemodynamic response function). The gain, loss and neutral outcome periods were modelled as regressors of no interest. The end fixation period of the task served as the implicit baseline.

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Registration was conducted through a two-step procedure, whereby EPI images were first registered to the high-resolution T1 structural image, then into standard (Montreal Neurological Institute, MNI avg152 template) space, with 12-parameter affine transformations. Higher-level (within group one-sample t-tests and between-group independent t-tests) analyses were conducted using FLAME (FMRIB's Local Analysis of Mixed Effects) on the gain, loss and neutral anticipation conditions described above. Significant clusters across the whole brain were determined by thresholding at $Z>2.3$ with a corrected (FWE) cluster significance threshold of $p<0.05$.

Time series and correlation matrices

Using FSL FEAT, we also separately modelled every individual gain, loss and neutral anticipation epoch within the context of the general linear model. For each MID run, this analysis yielded a total of 9 unique beta value images for each of the gain, loss and neutral anticipation conditions. Thus, each voxel-wise beta value image reflected the magnitude of the hemodynamic response evoked by each of the gain, loss and neutral anticipation epochs. Each beta value image for each MID run was then registered into standard (MNI avg152 template) space before being concatenated to generate a beta value "trial-wise" (e.g., gain anticipation) time series. Each beta value trial-wise time series for each MID run was then further concatenated across runs to generate a single beta value trial-wise time series for each of the MID anticipation conditions. This procedure yielded a 27 beta value trial-wise time series for the gain, loss and neutral anticipation conditions for each participant. This beta value trial-wise time series method has been previously employed to examine connectivity during distinct stages of different cognitive tasks

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(Fornito et al., 2011; Ray et al., 2017), including the MID task (Verdejo-Roman et al., 2017). Owing to the small, variable (performance-dependent) number of events for the outcome periods ("hits" and "misses") for each participant, we did not generate beta value trial-wise time series data sets for the network analyses.

Using the Harvard-Oxford atlas (96 cortical and 14 subcortical nodes/regions) as our connectome, we extracted the mean beta value time series from each of the 110 anatomical regions of interest (ROI) for the trial-wise gain, loss and neutral anticipation time series, for each participant. Using these mean ROI time series outputs, we conducted Pearson correlation coefficient analyses to construct whole brain ROI-to-ROI pairwise matrices (C*ij*). Each C*ij* was made up of 5995 (=N*(N − 1)/2, with N = 110 nodes) pairwise connections (edges). The C*ij* were used to estimate graph measures for the gain, loss and neutral anticipation conditions in each participant in the CAN and CON groups (see Supplementary Fig 2). The C*ij* were generated in MATLAB (The MathWorks, Inc., Natick, Massachusetts, United States).

Graph theory measures

Global (characteristic path length, efficiency and clustering coefficient) graph measures were estimated from each C*ij* using the GraphVar (www.rfmri.org/GraphVar) toolbox for functional brain connectivity (Kruschwitz et al., 2015) in MATLAB (The MathWorks, Inc., Natick, Massachusetts, United States). Comprehensive details of graph theory measures in brain networks can be found elsewhere (Bullmore et al., 2009a), but a brief description of our metrics will be provided here. Characteristic path length (L_p) is the minimum number of edges that must be traversed to go from one node (brain region) to another in a network. For a

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pair of nodes that are nearest neighbours, the path length is 1. Global efficiency (E_{glob}) describes how effective information flow is in the network and is inversely related to path length. Both these measures, ultimately, describe how well integrated the network is with respect to information exchange. The clustering coefficient (C_p), by contrast, quantifies the number of connections that exist between the nearest neighbours of a node, and describes how segregated the network is - the cliquishness of the network. L_p, E_{glob} and C_p measures are first estimated at each node of the connectome before an average (global value) is computed for the entire connectome. We additionally estimated the global metric of "small-world" propensity (Φ), which provides an unbiased assessment of "small-world" tendency in networks of varying densities (Muldoon et al., 2016). The "small-world" index combines a high C_p in addition to a short L_p (Watts et al., 1998) - there are clusters of nodes in the network that are also linked by short paths that enable efficient communication between clusters.

The above graph measures for each participant were estimated by thresholding each C*ij* at a selection of proportional cost (*K*) thresholds - thresholds that retain a percentage of the strongest connections (edges) in the network. This procedure was performed as it is argued that biological networks are represented by sparse connections (Latora et al., 2003), and that thresholding is a necessary step for the derivation of graphs to extract the appropriate topological properties of networks (Achard et al., 2007). Because graph measures can be sensitive to threshold value (van Wijk et al., 2010), however, we have reported our measures across a range of *K* thresholds ($0.1 \leqslant K \leqslant 0.5$, increments of 0.1). Here *K* represents the percentage (e.g., 0.1=10%) number of edges in each C*ij* that are

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maintained. We used a range of thresholds that represent the lower and upper bound of a small-world regime (Achard et al., 2007; Bullmore et al., 2011), that preserve the strongest functional connections for efficient parallel information processing at a relatively low wiring cost (Latora et al., 2001). All graph measures were computed from C*ij* in their weighted form following this thresholding procedure.

Functional connectivity

Group comparisons in ROI-to-ROI connectivity across C*ij* were additionally assessed using the Networks Based Statistics (NBS) Toolbox (Zalesky et al., 2010) for MATLAB (The MathWorks, Inc., Natick, Massachusetts, United States). These group comparisons were conducted to identify potential sub-components across the connectome where CON and CAN groups differ in connectivity strength. For each of the anticipation conditions, an independent groups t-test was first performed to test for a between-group difference in the correlation coefficients at each of the 110x(110-1)/2=5995 regional pairings. Graph sub-components were identified among the connections using a t-statistic threshold $t>3.1$. From here, a family-wise error (FWE) corrected *p*-value was calculated for the size of each resulting component using permutation testing (5000 permutations). Two (CAN < CON and CAN > CON) analyses were conducted independently on the gain, loss and neutral anticipation conditions. NBS has previously been used to explore connectivity strength between nodes of a connectome while psychiatric populations perform cognitive tasks (Fornito et al., 2011; Ray et al., 2017).

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Network visualisation

Networks that emerged from group comparisons conducted in NBS were visualised using the Brain Net Viewer (www.nitrc.org/projects/bnv/) software package (Xia et al., 2013) for MATLAB (The MathWorks, Inc., Natick, Massachusetts, United States).

Other Statistics

Group demographics were compared using simple independent t-tests. For analyses conducted on the MID behavioural data, we performed two (Group: CON vs. CAN) by three (Condition: Gain vs. Loss vs. Neutral) univariate analyses of variance. We also conducted independent t-tests on an index of the relative motivational value (RMV) for the gain and loss trials. This value is based on the ratio of percentage accuracy on the gain and loss trials compared to that on the neutral trials - i.e. Accuracy $_{gain/loss}$/Accuracy $_{neutral}$. Here a value >1 reflects a higher relative value of gain and loss incentives. We conducted two (Group: CON vs. CAN) by five ($0.1 \leqslant K \leqslant 0.5$) univariate analyses on the four global graph dependent variables, separately for the gain, loss and neutral anticipation conditions. For these analyses we invoked a Bonferroni correction ($0.05/N$, with $N = 12$ tests) to correct for multiple comparisons at a $p<0.004$ threshold. Therefore, only group effects with $p<0.004$ are reported in the results section for the graph measures. To test for potential differences in movement during scanning (mean mm absolute displacement), we conducted a two (Group: CON vs. CAN) by three (Run: run 1 vs. run 2 vs. run 3) repeated measures analyses of variance. Pearson correlation analyses were also conducted to test for associations between cannabis use (estimated life-time cannabis joints, age onset of use) and graph estimates (mean of *K* thresholds), but

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only where the CON and CAN groups were found to significantly differ. As these correlation analyses were more exploratory, we did not protect against a potential type 1 error by correcting for multiple comparisons. Estimated life-time cannabis joints values were Log (10) transformed to eliminate positive skew. All analyses were conducted using the Statistical Package for the Social Sciences (SPSS Inc., Chicago).

Results

Demographics

Table 1 (supplementary section) shows the demographic and substance use measures for the CON and CAN groups. The groups were balanced for gender and age, but did significantly differ on a number of measures, particularly alcohol, cigarette and illicit drug use, where the CAN group were significantly higher. The CAN group additionally had significantly lower mean WRAT score.

MID Performance

A two (Group: CON vs. CAN) by three (Condition: Gain vs. Loss vs. Neutral) analysis of variance showed that there was a significant effect of condition (F=4.9; df=104, 2; p<0.05; Gain>Neutral), and group (F=9.3; df=104, 1; p<0.01; CAN>CON), but no condition x group interaction (F=0.001; df=104, 2; p>0.05) for MID accuracy (see Supplementary Fig 3a). The same analysis showed a significant effect of condition (F=7.8; df=104, 2; p<0.01; Gain<Neutral), but no effect of group (F=0.09; df=104, 1; p>0.05), and no condition x group interaction (F=0.005; df=104, 2; p>0.05) for mean MID reaction time (see Supplementary Fig 3b). Independent t-test analyses, however, did not reveal any significant group differences for the loss

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(t = 0. 5, df = 34, p>0.05) or gain (t = 0.60, df = 34, p>0.05) rmv (see Supplementary Fig 3c), appearing to suggest that the two groups were well matched on the relative value of gain and loss incentives.

Functional MRI

There was no effect of run (F = 0.82; df = 2, 33; p>0.05), group (F = 0.14; df = 1, 34; p>0.05), or run x group interaction (F = 0.81; df = 2, 33; p>0.05) for motion during the MID task (CON 0.38 mm ± 0.06; CAN 0.42 mm ± 0.07). Mixed effects cluster-based one-sample t-test analyses showed that both the CON and CAN groups activated a predominantly fronto-striatal network of regions for all three anticipation periods (see Supplementary Fig 4). Mixed effects cluster-based independent t-test analyses, however, did not detect any significant differences between the two groups on these fMRI measures.

Graph theory results

The results from all univariate statistical tests conducted can be viewed in the supplementary results section. Here we report only the main effects of group that were detected for analyses on the graph measures. Two (Group: CON vs. CAN) by five ($0.1 \leqslant K \leqslant 0.5$) univariate analyses revealed a significant effect of group for gain anticipation C_p (F=17.13; df=170, 1; p<0.001; CAN>CON - Fig 1a), L_p, (F=12.35; df=170, 1; p=0.001; CAN<CON - Fig 1b), and E_{glob} (F=20.20; df=170, 1; p<0.001; CAN>CON - Fig 1c). There were no significant interactions indicating that these group differences were not sensitive to the specific K threshold that was employed. Supplementary Figure 5 also shows the mean of the K thresholds for the

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three measures where the groups significantly differed. There were no group differences on the graph measures for the loss and neutral anticipation conditions.

-Insert Figure 1 about here-

Functional connectivity results

Using NBS to test for group differences on the C*ij*, and to probe which nodes and connections could be driving the observed global graph differences, we detected a graph sub-network comprising 63 edges between 47 nodes where the CAN group demonstrated significantly greater connectivity strength ($p<0.05$) compared to the CON group during the gain anticipation period. This sub-network was made up of a number of nodes, including the amygdala, nucleus accumbens, hippocampus, insula, OFC, temporal, and lateral and medial pre-frontal cortical regions (Fig 2). There were no significant group differences for the loss or neutral anticipation periods.

-Insert Figure 2 about here-

Correlations

There were a number of correlations observed in the CAN group. The reported age of onset of cannabis use was found to be significantly correlated with C_p (r(16)=0.65, $p<0.01$ - Fig 3a), L_p (r(16)=-0.61, $p<0.01$ - Fig 3b) and E_{glob} (r(16)=0.72, $p<0.001$ - Fig 3c) during the gain anticipation condition. These correlations suggest that these measures of network efficiency are enhanced by the

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latency of cannabis use onset in the CAN group. There were no significant correlations between life-time cannabis joints and the graph measures.

-Insert Figure 3 about here-

Discussion

The present study compared the behavioural and neural correlates of gain, loss and neutral stimulus processing in an acutely abstinent adolescent cannabis-dependent (CAN) group against a control (CON) group using an MID task. Behaviourally, we observed significant performance differences between the two adolescent groups, where the CAN group demonstrated superior accuracy across all trial types. Additional analyses, however, revealed that the two groups appeared to be well matched on the metrics of loss and gain relative motivational value. Therefore, it is unclear whether the observed difference across all trial types is a reflection of more motivated behaviour in the CAN group, or merely superior psychomotor performance.

We also report an absence in group differences with respect to activation within neural systems during the gain, loss and neutral anticipation periods. This result, particularly with respect to gain anticipation does not appear to corroborate previous findings in cannabis users that have demonstrated hyperactivity within regions that underlie reward processing (Filbey et al., 2013a; Jager et al., 2013; Nestor et al., 2010). The CAN group did, however, demonstrate differences in global network topology and connectivity strength during the gain anticipation period that suggest differences in reward-associated connectivity. The group differences in global network topology appeared to be well correlated with the latency of cannabis use age onset in the CAN group. We discuss how these functional characteristics of

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brain connectivity may point to an enhanced level of network processing efficiency in adolescent cannabis dependence.

Increased network integration and segregation in the adolescent CAN group

The current study reports that during monetary gain anticipation the CAN group demonstrated enhanced E_{glob} and reduced L_p compared to the CON group. These global measures of integration appear to suggest a higher level of general information exchange across nodes of a brain network associated with reward processing. These measures of information transfer are not specific to any node or sub-network, but instead demonstrate differences across a network at varying connection densities, that appear to represent superior processing efficiency. This increase in processing efficiency appears to be in contrast to that reported in other addiction populations during rest (Holla et al., 2017; Wang et al., 2015). The CAN group additionally expressed enhanced C_p across the network during monetary gain anticipation. The C_p quantifies the number of connections that exist between the nearest neighbours of a node, and has been proposed as an index of local efficiency (Rubinov et al., 2010; Sporns et al., 2004). This apparent enhancement of local efficiency may suggest that there is more information processing within sub-networks of the brain in the CAN group that represents a tendency for neural segregation during reward processing. The markers of global efficiency and path length, by contrast, could indicate deficits in “neural refinement” (Gentili et al., 2015) that alternatively allude to “noisy” functioning across parts of the network (Prashad et al., 2018), while more clustered, local information processing may be a feature of less flexible information transfer across other parts. The observation of reduced L_p and concurrent increases in C_p might refute this possibility, however, and suggest

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more global processing efficiency through both segregated and connected network processing that is analogous to enhanced small-world propensity. While we did not observe significant groups differences on this global network metric, the CAN group did demonstrate an enhanced “small-world” signature compared to the CON group during the gain anticipation condition. This metric is characterized by increased interconnectivity and reduced path length between clusters of nodes (Bullmore et al., 2009b), indicating a concurrent efficiency of functional network segregation and integration (Achard et al., 2006; Salvador et al., 2005). The current findings could, however, be an indication of lower brain maturation in the CAN adolescent group. For example, there is evidence in adolescents with just one or two instances of cannabis use of significantly increased grey matter volume across the brain (Orr et al., 2019) that may be akin to cannabis-induced deficits in synaptic pruning. These deficits in typical maturation processes across the brain, could conceivably, be represented by increased connectivity across networks due to an increased number of synapses across nodes.

We further report that the age of cannabis use onset appeared to predict the enhancement of these network measures in the CAN group during reward anticipation. These correlations suggest that a delayed onset of cannabis use during adolescence was more strongly associated with processing efficiency across the network. This is contrary to what we would have expected in the CAN group. Distinct trajectories of cannabis exposure since initiation of use could conceivably have different consequences for the development of neural circuitry (Lichenstein et al., 2017) and implications for key psychological functions such as reward processing. Cannabis use shows a clear pattern of development, where initiation typically occurs during mid-teens and develops into a disorder between mid to late adolescence

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(Stinson et al., 2006). Sharp increases in cannabis exposure since the onset of use, for example, may challenge adaptation during the window of rapid neural development in adolescence (Kuhn et al., 2013). The observed associations reported here, therefore, could be a consequence of rapid escalations of, and dependence to, cannabis use since the initiation of use. This could have a sensitizing effect on brain networks that is represented through the enhanced segregated and integrated processing efficiency observed during reward processing. Alternatively, the observed correlations may reflect some "epiphenomenon", whereby the enhanced network functioning in the CAN group is actually a by-product less cannabis use.

Increased reward-related connectivity in the adolescent CAN group

We additionally report that the CAN group demonstrated greater connectivity strength across a sub-network of the connectome during the gain anticipation period. This network-based analysis revealed a total of 63 connections involving mostly frontal, temporal and subcortical nodes. This sub-network was made up of principally inter hemispheric connections between nodes, suggesting more long range routes of information exchange across the network during reward processing. Notably embedded in these inter hemispheric links were connections between the anterior parahippocampal gyrus (PHG) and orbitofrontal cortex (OFC). The PHG is implicated in the process of context appraisal (Kveraga et al., 2011), where in the present setting, expectations could be generated through contextual associative processing of reward cues. The OFC codes both the implicit motivational value (Rothkirch et al., 2012) and the incentive salience of a stimulus (Walter et al., 2010). Increased connectivity between these regions could represent enhanced contextual

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binding between cues and potential reward outcomes during anticipation. Intra hemispheric connections, while less prevalent, included links from the anterior PHG and the hippocampus to the NAcc, which may similarly represent a strengthening between circuits involved in memory and motivational processes. This sub-network, therefore, appears to show superior connectivity strength between nodes that integrate the cognitive, motivational and memory components of reward processing in the CAN group.

The presence of connectivity differences across a distributed sub-network of nodes, in the presence of group differences on graph topological measures of network efficiency in the same group, is notable. Previous studies (Cocchi et al., 2012; Fornito et al., 2011; Hong et al., 2013), using concomitant graph theory and network-based approaches, were not able to detect group differences using both these measures of network functioning. While topological and connectivity strength may be viewed as distinct measures, an apparent convergence of our findings across both analyses, in the same group, and on the same condition, may afford some level of fidelity to the current findings. The current findings, however, should be tempered with the possibility that reward-related connectivity differences across the network may, to some degree, pre-date cannabis use – they may have been a risk factor for cannabis (and other drug) use.

Limitations of the current study include an absence in matching of the adolescent CON and CAN groups with respect to WRAT scores, alcohol and cigarette use, anxiety, as well as recent illicit drug use. Strong correlations between covariates and independent variables, however, should be avoided (Suckling, 2011). There is currently no known statistical technique that can adequately account for such confounds, and the use of covariates (e.g., smoking) correlated with the

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independent variable (in this case, group) can lead to unpredictable results. Therefore, we did not use covariates in any of our analyses. We cannot, however, indisputably dismiss their potential influence on the network differences reported. Moreover, we did not collect data on the socioeconomic status of individuals in the CAN and CON groups, which if different, may have influenced behavioural and neural responses to financial rewards, which is indeed the very nature of the MID task. Furthermore, we did not assess cannabis craving and withdrawal in the CAN group, which may have had a disrupting effect on functional network connectivity. We cannot unequivocally dismiss the possibility of cannabis intoxication in the CAN group at the time of testing either, as we had no method of disqualifying this through means of toxicology. We also acknowledge that the current study involves small sample sizes in both groups, potentially curtailing the generalizability of our results to larger populations of cannabis-dependent and adolescent populations.

Conclusion

The period of adolescence is reflected by changes in brain networks that are likely to have significant effects on reward-seeking behaviours, and which are vulnerable to the onset of drug use. The current study has provided some preliminary evidence, albeit in a modest sample, that cannabis-dependent adolescents in acute abstinence show differences in global reward-associated network topology that are significantly correlated with cannabis use age onset. Further analyses show that there were differences in both intra and inter hemispheric connectivity strength across a distributed sub-network of nodes in the same group of cannabis-dependent adolescents. We propose that cannabis dependence in adolescence induces enhanced processing efficiency across a

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network during reward anticipation. This superior processing efficiency may be the product of sensitization, possibly through “accelerated” dependence. This sensitization may kindle the use of more harmful illicit drugs of abuse in adolescents with cannabis addiction.

Declaration of Conflicting Interests

The authors report no biomedical financial interests or potential conflicts of interest.

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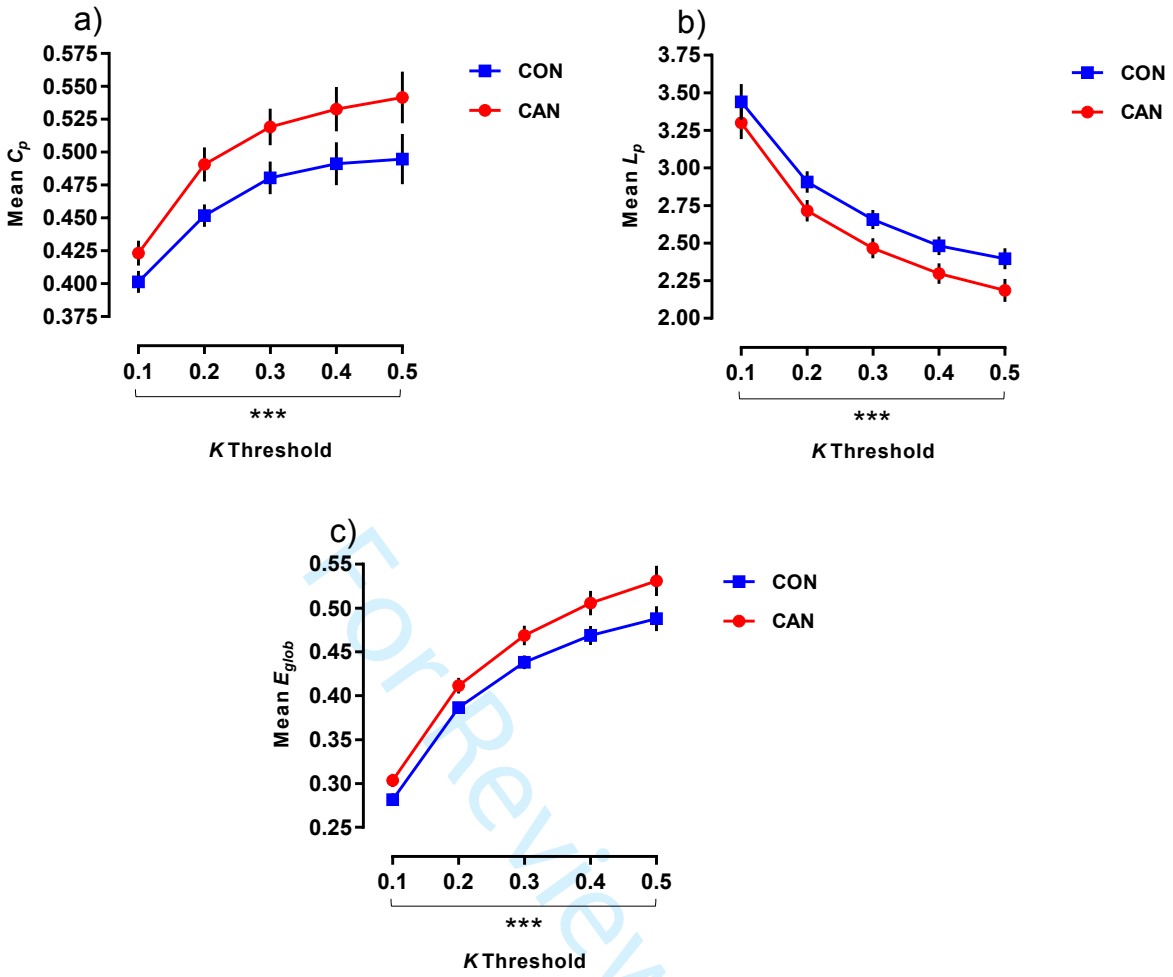

Figure 1. Group global network differences during the gain anticipation condition for a) C_p (***p<0.001, CAN>CON); b) L_p (***p<0.001, CAN<CON) and c) E_{glob} (***p<0.001, CAN>CON). Data were analyzed using two (group: CON vs. CAN) x five ($0.1 \leqslant K \leqslant 0.5$) univariate analyses. Data are expressed as means and standard errors.

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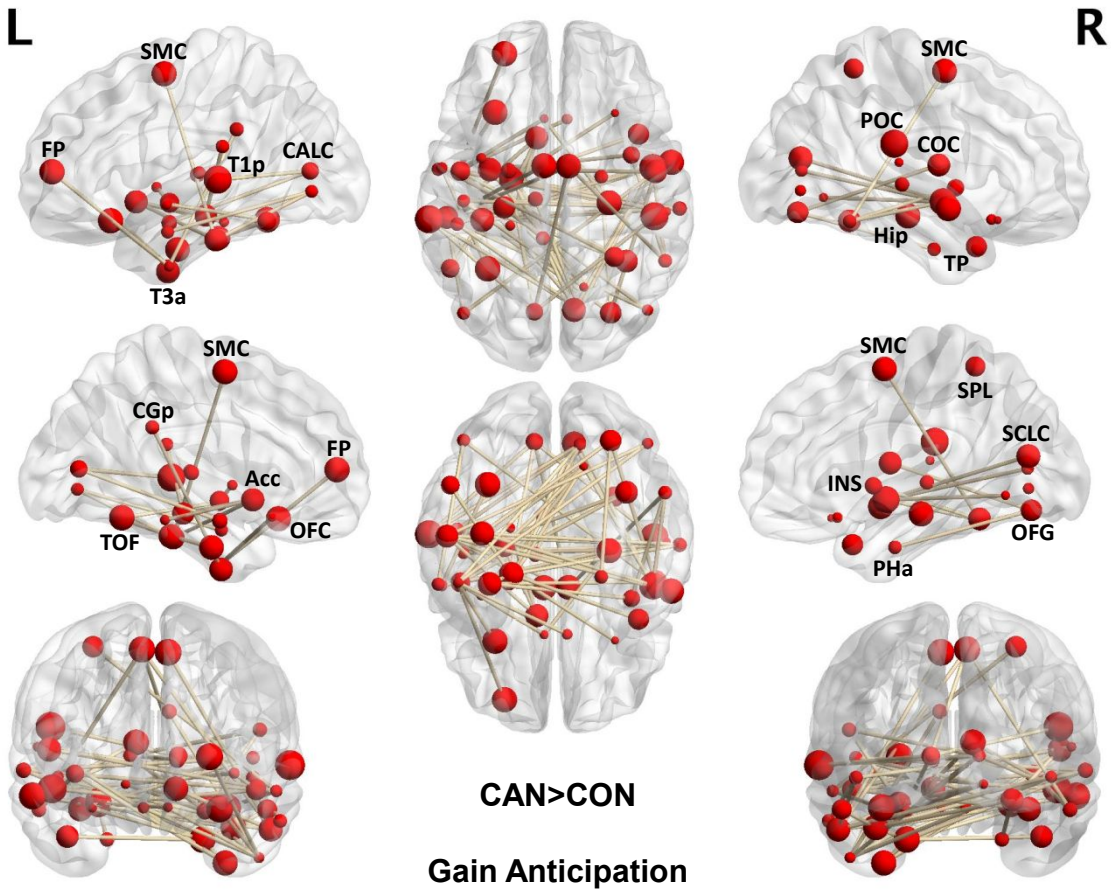

Figure 2. Network based statistics graph sub-component comprising 63 edges (p<0.05) where the adolescent CAN group demonstrated significantly greater connectivity strength compared to the adolescent CON group during the gain anticipation period. Graph sub-components were identified among all node pairwise connections with a t-statistic threshold of t > 3.1, corrected for multiple comparisons. L=left hemisphere; R=right hemisphere; Acc; nucleus accumbens; CALC=intracalcarine cortex; COC=central opercular cortex; CGp=posterior cingulate gyrus; FP=frontal pole; Hip=hippocampus; INS=insula; OFC=orbitofrontal cortex; OFG=occipital fusiform gyrus; PHa=parahippocampal gyrus, anterior division; POC=parietal opercular cortex; SCLC=supracalcarine cortex; SMC=supplementary motor cortex; SPL=superior parietal lobule; T1p=superior temporal ryrus, posterior division; T3a= inferior temporal gyrus, anterior division; TOF=temporal occipital fusiform cortex; TP=temporal pole.

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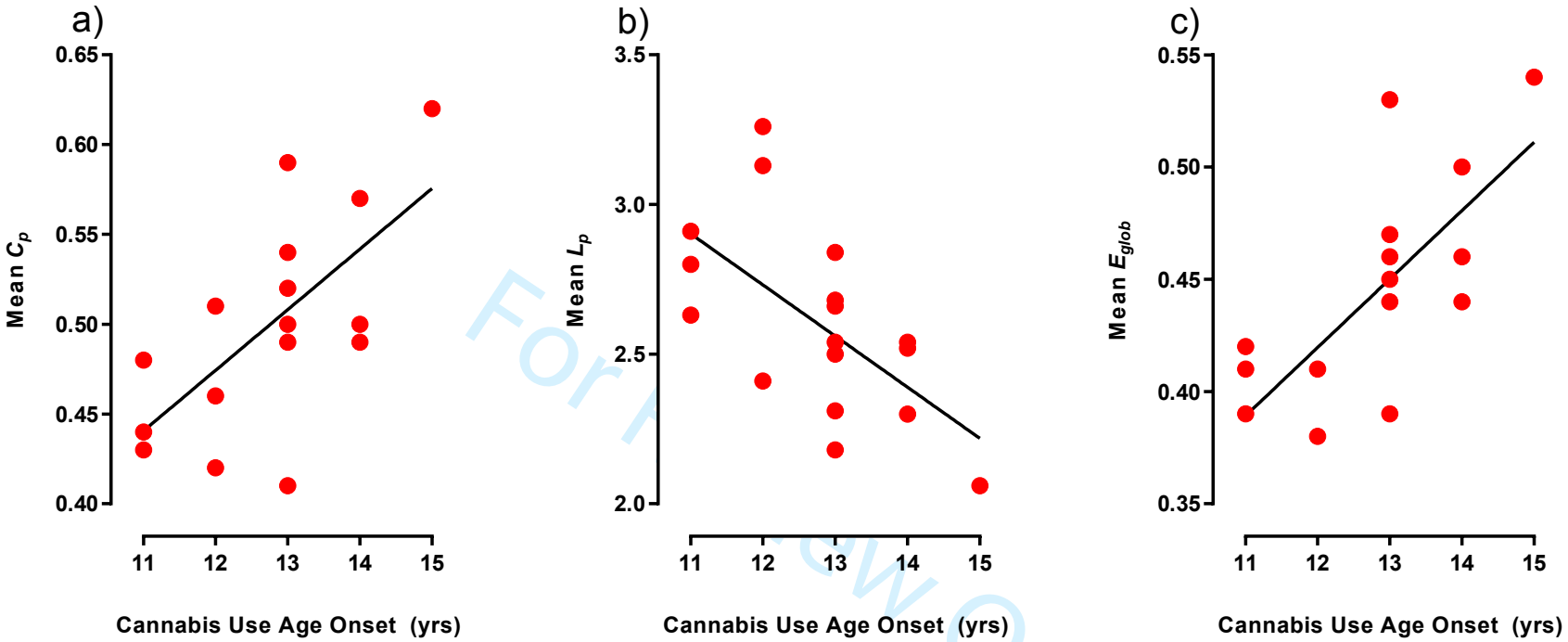

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Figure 3. Showing significant correlations in the adolescent CAN group between cannabis use age onset (yrs) and a) C_p (r(16)=0.65, p<0.01); b) L_p (r(16)=-0.61, p<0.01) and c) E_{glob} (r(16)=0.72, p<0.001) during the gain anticipation period.